

DEVELOPMENT OF EYE COLORS IN *DROSOPHILA*: PRODUCTION OF v^+ HORMONE BY FAT BODIES ¹

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Of the two diffusible substances known to be concerned in the production of eye pigments in *Drosophila*, only v^+ hormone is produced by fat bodies (Beadle, 1937). This was demonstrated by transplantation experiments. Attempts to extract this hormone from larval fat bodies were unsuccessful and it was therefore concluded that the hormone is produced after the time of puparium formation. It is the purpose of this paper to summarize additional experiments designed to determine when and under what conditions fat bodies produce this hormone. Unless otherwise indicated, fat bodies were taken from wild-type larvae or prepupae. All tests for v^+ hormone were made by using vermilion brown flies as described by Tatum and Beadle (1938). The few tests made for cn^+ hormone were made in a similar way using cinnabar brown flies.

LARVAL FAT BODIES

Although v^+ hormone could not be extracted from larval fat bodies with Ringer's solution at 100° C., it was felt that the hormone as such might nevertheless be present but in such a state that it was not extracted by the method used. Accordingly, several additional methods of extraction have been used.

Fifty sets of dissected fat bodies were heated in distilled water, oven-dried, and extracted with chloroform. The chloroform-insoluble material was taken up in hot Ringer's solution and injected into vermilion brown test larvae. The results were negative (8 flies). Since the hormone is known to be chloroform-insoluble and water-soluble, these tests confirm those previously made. Other tests in which the dissected fat bodies were ground with powdered silica were likewise negative (3 flies). Alternate freezing of fat bodies (in an acetone and solid CO₂ mixture) and thawing for six successive times failed to yield any hormone in subsequent extracts made with hot Ringer's solution (6 flies).

Digestion of larval fat bodies with trypsin failed to liberate any

¹ Work supported by funds granted by the Rockefeller Foundation.

hormone. In one experiment 20 sets of fat bodies from mature larvae were incubated for 24 hours at 37° C. in 0.03 cc. of a solution of 0.5 per cent of NaHCO_2 and 0.025 per cent of trypsin made up in Ringer's. The clear solution obtained after heating and centrifuging through a microfilter gave negative results (20 flies). Appropriate controls showed that under these conditions the trypsin used was active in digesting casein and that it did not alter the activity of concentrated extracts of the hormone. Observations showed that the trypsin-treated fat bodies were visibly broken down.

Further attempts to determine whether any v^+ hormone is present in larval fat bodies were made by freezing such tissues with solid CO_2 and then transplanting them to test animals. This was done by taking up the fat body tissue in a regular transplantation pipette (Ephrussi and Beadle, 1936) and then placing the shaft of the pipette in contact with a small piece of solid CO_2 . The temperature actually attained by the tissue itself was not determined; it was without question well above that of the CO_2 . Ten flies to each of which a section of fat body (attached along one margin to the salivary gland) had been transplanted after being frozen three times, showed little or no eye-color modification. Other experiments using a more or less similar technique were made with fat bodies immersed in boiling water before transplantation. Considerable difficulty was encountered in doing this, but by coating the inside of the pipettes with a thin film of agar, drawing up the fat-body tissue, and then immersing the pipette in boiling water, a number of successful transplants were made. Of seven test animals to which such heated fat bodies were transplanted, six were quite negative. The seventh showed a color modification of 2.5 (medium response—see Tatum and Beadle, 1938, for significance of color values). This exceptional animal was undoubtedly one to which by mistake an unheated fat body had been transplanted. Because of the technical difficulty of making such transplants such an error could easily have been made. Living fat bodies were transplanted as controls for both the frozen and heated series. Eight such control transplants gave a mean color value of 2.4 (1.8 to 3.0). A single control transplant gave a negative test, presumably due to failure of the operation.

These experiments agree with those previously made and indicate that little or no v^+ hormone is present in larval fat bodies prior to puparium formation, and consequently that the major portion of the hormone produced by such tissues is elaborated after puparium formation. It is possible that a small amount of hormone is produced before this time but in too small an amount to be detected by the methods used.

FAT BODIES OF PREPUPAE

Preliminary experiments indicated that v^+ hormone is present in prepupal fat bodies and can be extracted from them during this stage. Several series of extractions of prepupal fat bodies taken from animals of various ages were therefore made. In each case 20 sets of fat bodies were heated in 0.03 cc. of Ringer's solution and the solution removed by centrifuging through a microfilter. One series, using prepupae from the Oregon-r wild-type stock gave the results shown in Table I.

TABLE I

Age in Hours after Puparium Formation	Number of Test Animals	Eye Color, Mean and Range
0-1	11	0.1 (0.0-0.4)
3-4.5	14	0.0
6-8	10	2.2 (1.5-3.0)
7-9	15	2.3 (0.0-3.2)
10-11.8	10	0.8 (0.0-2.7)

There is no apparent reason why the 3-4.5 hour prepupal fat bodies gave negative results. A number of other tests indicate that the results are generally erratic for young prepupae. Thus a separate set gave a mean color value of 1.3 for an extract of 0-1 hour prepupal fat bodies. A series of tests of prepupae from the Canton-S wild-type stock gave the results shown in Table II.

TABLE II

Age in hours	Number	Eye Color
larval	10	0.0
0-1	10	0.3 (0.1-0.6)
1.8-3.5	8	0.7 (0.0-1.6)
8-9.5	8	1.4 (0.7-2.0)

An additional experiment using 10-12-hour Oregon-r prepupae gave a test of 2.3 (6 animals 1.3-3.0). It should be pointed out that the tests of older prepupae are unreliable because of the impossibility of being sure of getting all of the fat body tissue. At this time the fat bodies are undergoing the breakdown process characteristic of metamorphosis.

These results suffice to show that the hormone is present in fat body tissue and may be extracted over most of the prepupal period. Because of the several difficulties involved in such tests as these, the results are only roughly indicative of quantitative relations.



CORRELATION OF HORMONE PRODUCTION AND PUPARIUM FORMATION

Various attempts have been made to alter the conditions so that larval fat bodies would produce v^+ hormone. Unheated wild-type larval fat bodies were allowed to stand in Ringer's solution for 5 to 6 hours at room temperature. Extracts of these gave negative results. Several series of 48-hour-old wild-type larvae were subjected to semi-starvation conditions by transferring them to 0.25 per cent dry brewers' yeast in 1 per cent agar as described by Beadle, Tatum and Clancy (1938). This reduced food supply prolongs larval life. Extracts of the fat bodies of such delayed larvae made just prior to puparium formation failed to show the presence of hormone. On the assumption that enzymes might be involved in the production of v^+ hormone by the fat bodies, pupal fluid from vermilion brown animals selected from 0 to 30 hours after puparium formation was injected into wild-type larvae 117–124 hours after egg-laying. Three to 7 hours after these injections were made the fat bodies of the hosts were removed and extracted with hot Ringer's. These extracts were negative in tests for v^+ hormone. A similar experiment in which vermilion brown pupal fluid was injected into 92–97-hour wild-type larvae gave negative results in tests of fat body extracts made 23–25 hours later.

A marked delay in puparium formation brought about by subjecting mature larvae to low temperature apparently does not break down the synchronism between hormone production and puparium formation. An experiment in which wild-type mature larvae were kept at 8–10° C. for 18.5 hours showed that a Ringer extract of fat bodies of 0–1-hour-old prepupae taken at the end of this time gave a mean color value of 0.3 when tested in 11 vermilion brown animals. A comparable extract made from prepupal fat bodies from mature larvae kept continuously at 25° C. gave an average color value of 0.6 (11 flies). Considering the low values obtained from these two extracts and the variation (0.1–0.7 and 0.1 to 0.8 respectively), this difference cannot be regarded as significant.

Prepupal fat bodies 0–1 hours after puparium formation apparently do not continue hormone production when explanted to Ringer's solution. In one experiment 20 sets of such fat bodies were placed in 0.03 cc. of Ringer's solution and allowed to stand at 22° C. for 27–28 hours. At the end of this time a hot-Ringer extract gave a color value of 0.4 (range 0.0–0.7, 14 animals). A control series extracted in a similar way immediately on dissection gave a color value of 0.6 (range 0.1–0.8, 11 animals). The explanted fat bodies did not undergo the breakdown processes characteristic of metamorphosis.

Superfemales of *Drosophila* (individuals with 3 X chromosomes and 2 sets of autosomes) are known to show a delay of one to three days in puparium formation as compared with their normal sisters (Brehme, 1937). During this period, subsequent to puparium formation by their sibs, there is relatively little growth of the superfemale larvae. Extracts of fat bodies of such superfemale larvae taken shortly before puparium formation show that v^+ hormone is present at this time. Thus an extract of 20 sets of fat bodies from mature superfemale larvae in 0.03 cc. of Ringer's solution gave a mean eye-color modification of 0.7 (range 0.0–1.0, 10 animals). Extracts of prepupal fat bodies of superfemales are likewise positive. It is clear, then, that under the particular set of developmental conditions of superfemale larvae the synchronization of fat-body hormone production with puparium formation characteristic of normal larvae is broken down. This shows that the two processes are not inseparably associated at least as regards their time sequence. The mechanism by which the two processes are normally related, however, is entirely a matter of conjecture at the present time.

While under none of the environmental and experimental conditions to which normal larvae were subjected was there any appreciable production of v^+ hormone by the fat-body cells prior to puparium formation, the fact that the sequence of these two processes is modified by the genic imbalance characteristic of superfemales suggests that it might be possible to induce the formation of hormone by cells of this tissue before puparium formation in normal larvae if the proper conditions were brought about. Certainly this possibility is not excluded by any of the work reported in this paper.

In order to determine whether or not fat bodies might have any effect on the eye-color hormones *in vitro*, an experiment was made in which fat bodies were explanted to a Ringer's solution containing partially purified v^+ and cn^+ hormones. As a control, fat bodies heated for several seconds at 100° C. were allowed to stand in a similar solution of the hormones. In both cases the fat bodies were kept in the solution for 4 hours at room temperature. The results are shown in Table III.

Living fat bodies appear to have no significant effect on the hormones in solution. Since the hormones may be inactivated through oxidation in the presence of certain enzymes present in the organism (Thimann and Beadle, 1937), it may be concluded that the fat body either does not contain or does not liberate such enzymes under the conditions of this experiment.

RELATION OF THE FAT BODY TO THE STARVATION EFFECT

It has been shown that low food level at a certain period of development modifies vermilion flies in some manner such that they produce v^+ hormone (Khouvine, Ephrussi and Chevais, 1938; Beadle, Tatum and Clancy, 1938). Normally such flies produce little or no v^+ eye-color hormone. Since this modification evidently must be due to some alteration in metabolism, attempts have been made to determine what tissues or organs might be involved. It has been found that the fat body is modified by subjection of larvae to low food.

Larvae were transferred from full food to low food at about 48 hours after egg-laying and allowed to complete larval development under these conditions. The methods of inducing an eye color modification in this way are described in the papers referred to above. Fat bodies taken from mature larvae which had been subjected to such semi-starvation conditions were transplanted to vermilion brown larval hosts

TABLE III

	Test for v^+ hormone		Test for cn^+ hormone	
	Unheated	Heated	Unheated	Heated
Number of tests.....	11	11	8	9
Mean eye color.....	3.0	3.1	2.5	2.4
Range.....	2-3.5	3.0-3.5	2.5	2.0-2.5

grown under standard full-food conditions. In one experiment in which fat bodies from vermilion brown larvae grown on low food were transplanted, 21 host animals eclosed. Of these, 16 showed an eye-color modification (mean 0.8, range 0.1-2.0). The remaining 5 were negative, possibly because of unsuccessful operations. Since the fat body normally breaks down during metamorphosis there is no easy way of checking for the presence of implanted tissue. In another series fat bodies from vermilion larvae subjected to a low food level were transplanted to vermilion brown test larvae. Ten animals developed and all showed a positive effect of the implant (mean eye color 1.3, range 0.8-1.9).

Since it is well established that fat bodies of fully fed vermilion (or vermilion brown) larvae give negative results when transplanted to vermilion brown hosts, it is evident that low food of the kind used so modifies the fat body that it subsequently produces v^+ hormone. These results have been checked by direct extraction of the hormone from pre-pupal fat bodies. Extraction of fat bodies of mature vermilion brown

larvae that had been subjected to low food conditions yielded solutions that were negative in tests for v^+ hormone. Fat bodies from prepupae (4.5–6.5 hours after puparium formation) were extracted with hot Ringer's solution. This extract gave a slight but definitely positive modification of the eyes of vermilion brown test animals (11 flies, eye color 0.1–0.2). It appears that in such larvae, as in normal wild-type larvae, the fat body produces v^+ hormone subsequent to the time of puparium formation.

Preliminary studies have indicated that subjection of larvae to low food conditions brings about changes in the cytoplasmic inclusions of the fat body cells. These changes may possibly be correlated with the production of hormone by vermilion larvae which have been grown under semi-starvation conditions. Since these investigations are as yet incomplete, discussion of them will be deferred.

Malpighian tubes of wild-type larvae are known to contain v^+ hormone and there is evidence that they produce this substance. In order to determine whether the low food level might also have an effect on these organs, Malpighian tubes from semi-starved vermilion brown (or vermilion) larvae were transplanted to normal vermilion brown test larvae. It was discovered that tubes from larvae subjected to a low food level tend to kill the hosts to which they are transplanted. Presumably the tubes accumulate toxic substances under such conditions. In a preliminary series four mature recipients showed no eye color modification. In this series, however, no dissections were made to determine whether the implant was present. A second series in which sets of four Malpighian tubes from mature semi-starved vermilion were transplanted to vermilion brown test larvae, nine adult recipients were obtained which dissections showed to contain implanted tubes. Eight of these showed a relatively weak eye color response (0.5) indicating that hormone was present or was produced—the ninth was negative.

It appears that the Malpighian tubes of vermilion larvae contain or produce some v^+ hormone under the semi-starvation conditions to which these larvae were subjected. The effect, however, seems to be less strong than that on the fat bodies. It is possible that the hormone released from larval Malpighian tube transplants represents accumulation and is not produced by the tubes themselves. It does not seem probable that the hormone is produced by the fat body, although we have not entirely excluded the possibility that the larval fat body produces hormone at a low rate. The fact that no hormone (or very little) accumulates in the larval fat body argues that if it is produced in this tissue during larval life, it must diffuse out approximately as fast as it is formed.



SUMMARY

Under normal genetic and environmental conditions fat-body cells produce v^+ hormone after the time of puparium formation but not before. Attempts to induce hormone production by fat-body tissue before puparium formation were unsuccessful. Since it is shown that larval fat bodies of mature superfemale larvae contain v^+ hormone, however, it is clear that the normal sequence of puparium formation and hormone production is not a necessary and invariable one.

Active solutions of v^+ hormone are readily obtained by extracting prepupal fat bodies over practically the entire period of prepupal development.

It is shown that the so-called "starvation effect" on eye pigmentation involves a modification of genetically vermilion fat body cells such that they produce v^+ hormone, whereas normally they are unable to do so. It is possible but not definitely established that a somewhat similar modification is brought about in cells of the Malpighian tubes by semi-starvation of larvae.

LITERATURE CITED

- BEADLE, G. W., 1937. Development of eye colors in *Drosophila*: fat bodies and Malpighian tubes in relation to diffusible substances. *Genetics*, **22**: 587-611.
- BEADLE, G. W., E. L. TATUM, AND C. W. CLANCY, 1938. Food level in relation to rate of development and eye pigmentation in *Drosophila melanogaster*. *Biol. Bull.*, **75**: 447-462.
- BREHME, K. S., 1937. Effects of the triplo-X condition on development in *Drosophila melanogaster*. *Proc. Soc. Exper. Biol. and Med.*, **37**: 578-580.
- EPHRUSSI, B., AND G. W. BEADLE, 1936. A technique of transplantation for *Drosophila*. *Am. Nat.*, **70**: 218-225.
- KHOUVINE, Y., B. EPHRUSSI, AND S. CHEVAIS, 1938. Development of eye colors in *Drosophila*: nature of the diffusible substances; effects of yeast, peptones and starvation on their production. *Biol. Bull.*, **75**: 425-446.
- TATUM, E. L., AND G. W. BEADLE, 1938. Development of eye colors in *Drosophila*: some properties of the hormones concerned. *Jour. Gen. Physiol.*, **22**: 239-253.
- THIMANN, K. V., AND G. W. BEADLE, 1937. Development of eye colors in *Drosophila*: extraction of the diffusible substances concerned. *Proc. Nat. Acad. Sci.*, **23**: 143-146.